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Transformability of *galE* Variants Derived from Uropathogenic *Escherichia coli* Strains

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Transformation of uropathogenic *Escherichia coli* strains with plasmid DNA was in general unsuccessful or very inefficient. Transformation was much more efficient when *galE* mutants of such strains, in which the lipopolysaccharide chains appeared shorter, were used as recipients.

In studies on structures and functions involved in the virulence of pathogenic *Escherichia coli* strains, molecular cloning and in vitro DNA manipulations of relevant genomic regions are successful approaches (1, 4, 7, 13, 14, 17). In such studies, transfer of cloned or manipulated genes to the pathogenic *E. coli* strains might be an essential step. Transformation is an obvious technique to accomplish this. For *E. coli* K-12, several transformation techniques, based on a Ca^{2+} plus heat shock treatment of cells, have been reported (2, 5, 9, 12).

These techniques have been used to transform a number of uropathogenic *E. coli* strains belonging to various serological classes with the vector plasmids pBR322 and pACYC184. The procedure of Dagert and Ehrlich (5) reproducibly gave the best results. This technique was used in further experiments. The results of the transformation of a number of uropathogenic strains with pBR322 DNA are shown in Table 1. Strains AD110, AD119, and Z094 gave very low numbers of transformants. The low efficiency obtained with these strains is representative for most uropathogenic and wild-type *E. coli* strains isolated from sewage that we tested (a total of 15 were tested). Strain AD403 was rather exceptional since it appeared to be readily transformable. Similar results were obtained when plasmid pACYC184 was used (data not shown). The low efficiency that we observed in plasmid transformation with most of our wild-type *E. coli* strains could be the result of low DNA uptake, of DNA restriction, of incompatibility between the plasmid and resident plasmids, or of a combination of any of these factors. Incompatibility is unlikely to be the main cause for the low efficiency, since we obtained comparably low yields with pBR322 and with pACYC184, plasmids that belong to different incompatibility groups. The low transformability is more likely caused by inefficient DNA uptake and by restriction.

In *E. coli* transformation, DNA uptake appears to occur when the physical state of the membrane lipids changes from the solid to the liquid phase during the heat shock applied in the transformation protocols (16). Probably it is a prerequisite that the transforming DNA is in intimate contact with the recipient cell envelope during this short period, as discussed recently (6). The long lipopolysaccharide (LPS) chains present on the surface of most wild-type *E. coli* strains might prevent this contact. It has indeed been reported (3) that rough variants of smooth *Salmonella typhimurium* organisms, especially *galE* mutants, showed increased transfectability compared with their smooth parental strains.

We therefore decide to isolate *galE* mutants of the three uropathogenic strains (Table 1) that showed low transformability. The *E. coli* strains were treated with the mutagenic agent ethyl methanesulfonate. After a treatment period that allowed a survival of 0.1 to 0.01% of CFU, the survivors were plated on peptone agar (10 g of Bacto-Peptone, 5 g of yeast extract, and 5 g of Na_2HPO_4 per liter) in such a dilution that single colonies appear. After growth at 37°C for 18 h, the cells were replica plated on peptone-agar medium supplemented with 6 g of galactose per liter. On such plates, *galK* and *galT* mutants show galactose-induced bacteriostosis, whereas *galE* mutants show bacteriolysis (19). Colonies that did not grow on the replica plates were further tested. Cells from such colonies were grown overnight in peptone broth and then diluted 1:100 in broth supplemented with galactose. Growth of the cells at 37°C in a shaking water bath was measured by means of a Klett-Summerson photometer. Typical *galE* mutants lysed after 2 h of incubation.

In all variants that behaved like a *galE* mutant, we tested the uridine diphosphogalactose-4-epimerase (epimerase) activity. Cells were grown in minimal medium with D-fucose as inducer for the galactose operon and glycerol as carbon source. Wet cells (1 g) were disintegrated in a French pressure cell. The epimerase activity in the extract was determined as described by Kalckar et al. (8). In all putative *galE* mutants, the epimerase activity was strongly reduced (to 1% or less). The transformation results (Table 1) clearly indicate that the *galE* mutants were more suitable for transformation than were their $galE^+$ parental strains. The effect of the *galE* mutation was most likely indirect, based on its effect on the LPS structure. To see what effect the *galE* mutation had on the LPS composition, LPS was isolated and analyzed. Parental strains and the *galE* mutants were grown overnight in minimal medium supplemented with 2 g of glucose per liter, diluted 1:100 in minimal medium, and cultivated in a shaking water bath for 2 h at 37°C. From approximately 0.3 g of cells (wet weight), cell envelopes

TABLE 1. Transformation frequency of uropathogenic *E. coli* strains and their *galE* mutants with pBR322 DNA

Strain	Serotype	No. of transformants per μg of pBR322 DNA propagated on:	
		E. coli K-12	Homologous *galE* mutant
AD110	O6:K2:H1	<1	15
AD110 *galE*		200	800
AD119	O18ac:K^+:H^-	3	80
AD119 *galE*		80	900
Z094	O87:K^-:H19	35	500
Z094 *galE*		1,000	7,000
AD403	O86	6,000	

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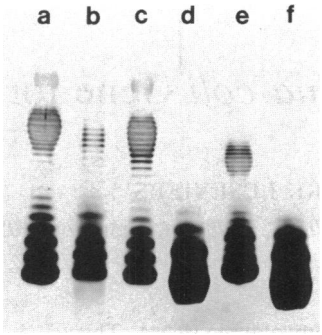

FIG. 1. Polyacrylamide gel analysis of LPS (15) isolated from (a) AD110, (b) AD110 *galE*, (c) AD119, (d) AD119 *galE*, (e) ZO94, and (f) ZO94 *galE*. The 2-keto-3-deoxyoctulosonic acid content of each LPS sample was determined (18) and, based on these figures, equal amounts were analyzed on the gel.

were isolated (11). To isolate the LPS, the cell envelopes were extracted with 2 ml of hot phenol as described previously (10). Analysis of equal amounts of the isolated LPS by a sodium dodecyl sulfate-polyacrylamide gel electrophoresis technique, described by Tsai and Frasch (15), revealed that all *galE* mutants had deficient LPS structures (Fig. 1). In the *galE* mutants of strains AD119 and Z094 there was a profound change in the length of the LPS chains. In strain AD110 there was only a slight reduction; whether this was the consequence of a basic level of epimerase still present in this strain is not known.

In the transformation of wild-type *E. coli* strains with plasmid DNA propagated in *E. coli* K-12, restriction might be another cause for the low transformability. With plasmid DNA isolated from the *galE* mutant, transformation of the relevant wild-type strain appeared to be 5 to 30 times more effective (Table 1). Apparently such DNA has the proper host modification and consequently is not affected by restriction. In situations in which it is necessary to transform wild-type *E. coli* cells, cloned DNA propagated in *E. coli* K-12 might be transferred first to a *galE* mutant of that wild-type strain to provide it with the proper host modification. Host-modified DNA can then be used to transform the wild-type strain. This strategy has its limitations: it was successful with the small vector plasmids used here (Table 1), but it did not work with strain AD110 when we used plasmids sized ca. 20 kilobases or more. We used recombinant plasmids sized 20 and 27 kilobases, and we obtained transformants in the *galE* mutant of strain AD110. However, with the plasmid DNA preparations isolated from these transformants, we did not obtain any transformants when AD110 was used as recipient. Apparently such DNA molecules were too large to be taken up by the wild-type parental strain.

We assume that the low efficiency for transformation of wild-type *E. coli* is caused primarily by shielding of entrance sites by the long LPS chains. Reduction of LPS chain length, as for example in *galE* mutants, increases the efficiency of DNA uptake. The use of the *galE* mutant as an intermediate host, moreover, might be helpful in overcoming the restriction barrier.

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